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None

(58) Field of search
UK CL (Edition K) C2C CBW CPN CTV
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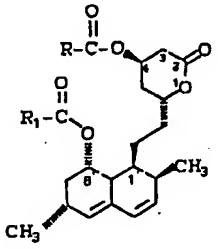
Online databases: CAS ONLINE

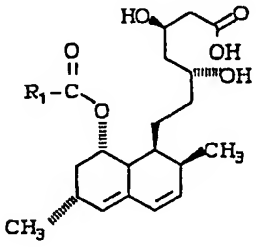
(54) Enzymatic deacylation of simvastatin

(57) A process is described for the enzymatic deacylation of compound (II)

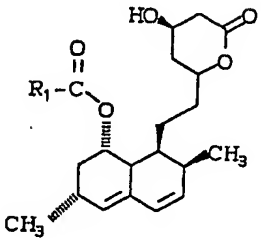


(II)

to form compounds of formula (III) and (IV):



(III)



(IV)

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TITLE OF INVENTION

ENZYMATIC DEACYLATION OF SIMVASTATIN

BACKGROUND OF THE INVENTION

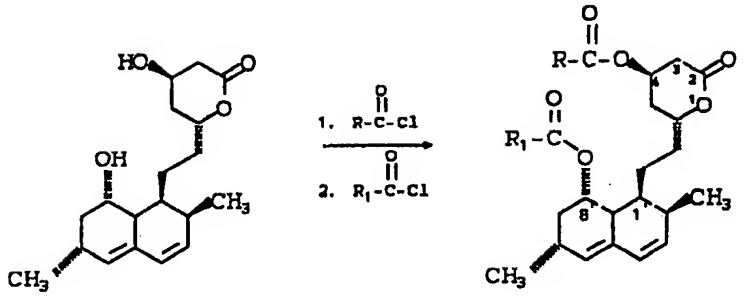
Hypercholesterolemia is known to be one of the prime risk factors for ischemic cardiovascular disease such as arteriosclerosis. Bile acid sequestrants have been used to treat this condition; they seem to be moderately effective but they must be consumed in large quantities, i.e. several grams at a time, and they are not very palatable.

MEVACOR® (lovastatin), now commercially available, is one of a group of very active antihypercholesterolemic agents that function by limiting cholesterol biosynthesis by inhibiting the enzyme HMG-CoA reductase. In addition to the natural fermentation products, mevastatin and lovastatin, there are a variety of semi synthetic and totally synthetic analogs thereof which also inhibit HMG-CoA reductase.

An important analog of lovastatin is simvastatin, now commercially available as ZOCOR®. Simvastatin may be formed from 8'-hydroxy-des-(α-methylbutyryl)-lovastatin which in turn is formed from lovastatin (U.S. Patent 4,784,444). One problem in this procedure is the need to block the 4-hydroxy moiety on the lactone ring and then, after insertion of the 8'-ester moiety, remove this lactone hydroxyl blocking group without affecting the 8'-ester group. It is particularly important that removal of the lactone hydroxyl blocking group take place efficiently and without the introduction of undersirable side products such as dehydrosimvastatin which can form as a result of the lability of the lactone ring to acid and base hydrolysis. The present invention provides a solution to the above problems with respect to the removal of an acyl blocking group at the lactone hydroxyl.

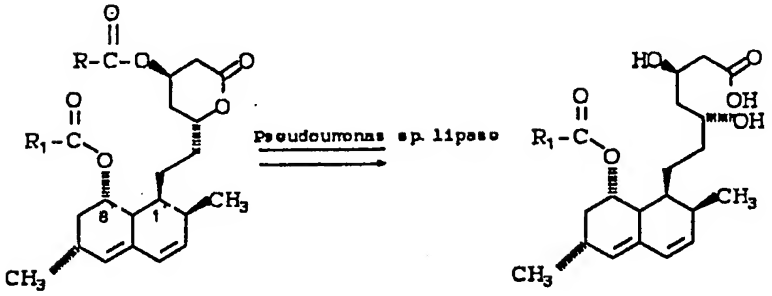
DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to a process for the enzymatic deacylation of a compound (II) to form a compound of structural formula (III) and its corresponding lactone of formula (IV).

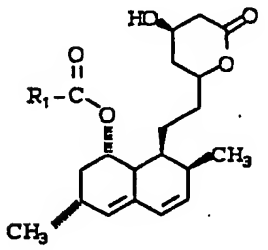


(I) (II)

Diol lactone



(II) (III)



(IV)

wherein:

R is C_{1-10}alkyl;

R_1 is C_{1-10}alkyl.

In one embodiment of this process
R is C_{1-5}alkyl; and
R_1 is C_{1-5}alkyl.

In a class of this embodiment R is selected from the group consisting of: methyl, propyl, isopropyl, or pentyl. R_1 is selected from the group consisting of 2-methyl-2-butyl or 2-butyl. The compound (III) wherein R_1 is 2-methyl-2-butyl is the open acid form of simvastatin.

The process disclosed herein involves the selective enzymatic deacylation at the 4-position of the lactone moiety leaving intact the 8'-acyl group on the polyhydronaphthyl ring. The hydroxy acid (III) may be converted to the its ammonium salt, a useful intermediate in a synthetic sequence to simvastatin. Copending application Attorney docket number 18305, the contents of which are hereby incorporated by reference, describes a synthetic sequence to simvastatin, which employs the ammonium salt of simvastatin. The present enzymatic deacylation process has distinct advantages over a chemical deacylation; the enzymatic process is simple to use, efficient and results in the products (III) and (IV) without the formation of complicating side products. Furthermore the process yields a water-soluble acid which can be used directly to form the ammonium salt.

The enzyme employed may be a commercially available lipase or esterase or one produced by fermentation. Sources of the enzyme are:

1. Pseudomonas sp. lipase (Sigma Chemical Co.)
2. LPL-80 Lipase (Amano Int'l. Enzyme Co.)
3. Pig liver esterase (Amano Int'l Enzyme Co.)
4. Candida cylindracia Lipase (Sigma Chemical Co.)
5. Supernatant from broth of Pseudomonas aeruginosa (MB 5001) (MB = Merck Bacteria)

Excretion and purification of the lipase from the both of Pseudomonas aeruginosa is described by W. Stuen et al. in J. Bacteriology 168(a), 1070 (1986).

The starting diol lactone (I) may be prepared following the procedures in U.S. Patent 4,293,496 and U.K. publication 2,075,013, The diol lactone is converted to the bisacylated material (II) in a sequence involving acylation of the lactone 4-position with the appropriate acyl halide or acid anhydride followed by esterification at the polyhydronaphthyl ring 8'-position using an appropriate acyl chloride or bromide, preferably 2,2-dimethylbutyryl chloride or bromide.

For the enzymatic deacylation, substrates are generally reacted with the enzyme in a solution of buffer with a pH range of 6.0 to 8.0. The substrates, which are water-immiscible, are dissolved in a water-miscible organic solvent and added to the enzyme-containing buffer solution. Incubation is carried out at from 22 to 40°C with or without agitation for a time period of up to 48 hours. Recovery of the substrate involves a simple organic extraction to remove the product; pH adjustment during the extraction may be necessary depending on the form of the product to be removed.

EXAMPLE 1

Preparation of 7-[1,2,6,7,8,8a(Ra)-hexahydro-2(S),6(R)-dimethyl-8(S)-(2,2-dimethylbutyryloxy)-1(S)-naphthyl]-3(R),5(R)-dihydroxyheptanoic acid and 6(R)-[2-[8(S)-(2,2-dimethylbutyryloxy)-2(S),6(R)-dimethyl-1,2,6,7,8,8a(R)-hexahydronaphthyl-1(S)]-ethyl-4(R)-hydroxy-3,4,5,6-tetrahydro-2H-pyran-2-one

(a) Preparation of 6(R)-[2-[8(S)-(hydroxy)-2(S),6(R)-dimethyl-1,2,6,7,8,8a(R)-hexahydro-naphthyl-1(S)]ethyl-4(R)-acetyl-3,4,5,6-tetrahydro-2H-pyran-2-one(1)

To crude dry diol lactone (I) (5.0g; 0.0156mol) and 4-dimethylamino pyridine (DMAP) (0.3813g; 0.0031mol; 20 mol%) in dry pyridine (30mL) at 0°C under nitrogen was added acetic anhydride (1.77mL; 0.017mol) in one shot and the mixture stirred for 4-6 hours at 0°C.

The pyridine was evaporated and ethyl acetate was added (60mL). The solution was washed with saturated NaCl (60mL), the layers were separated, and the organic layer dried over molecular sieves. The organic layer was filtered and the ethyl acetate was evaporated off to give a light brown solid.

Alternatively the reaction mixture could be recovered by addition of ethyl acetate (60 ml) followed by washing with saturated copper sulfate (4 x 50 ml), the two phases separated and the organic layer dried over anhydrous magnesium sulfate or

anhydrous sodium sulfate. The organic layer was filtered and the ethyl acetate was evaporated off to give a light brown solid.

(b) Preparation of 2,2-dimethylbutyryl chloride

To dimethylbutyric acid (24.04g; 0.207mol) at room temperature under nitrogen was added thionyl chloride (16.6mL; 0.227mol) and the mixture stirred for 5 hours. The product was distilled (29 in Hg) at 52-53°C to give a clear liquid.

(c) Preparation of 6(R)-[2-[8(S)-(2,2-dimethyl-butyryloxy)-2(S),6(R)-dimethyl-1,2,6,7,8,8a (R)-hexanhydronaphthyl-1(S)]ethyl-4(R)-acetyl-3,4,5,6-tetrahydro-2H-pyran-2-one(2)

The crude 4-acetylated diol lactone prepared in 1(a) above (3.15g; 0.0087mol) under nitrogen was dissolved in dry pyridine(8.7mL); DMAP (0.2126g; 0.0017mol; 20mol%) was added and the temperature was decreased to 0°C. 2,2-dimethylbutyryl chloride (9.37g; 0.0696mol; 8 equivalents) was added over 10 minutes and the mixture was stirred for 0.5 to 1 hour at this temperature. The reaction temperature was increased to 35 to 40°C and stirred for 48 hours.

The pyridine was evaporated and ethyl acetate (60 ml) was added. The solution was washed with saturated NaCl (20ml), the layers separated, and the organic layer dried over molecular sieves. The organic layer was filtered and the ethyl acetate evaporated to give a light brown solid.

Alternatively the above reaction mixture could be recovered by addition of ethyl acetate (60 ml) followed by washing with saturated copper sulfate (4 x 50 ml), separating the layers and drying the organic layer over anhydrous magnesium sulfate or anhydrous sodium sulfate. The organic layer was filtered and the ethyl acetate was evaporated off to give a light brown solid.

(d) Biotransformation

A. Culture Conditions and Bioconversion

A working culture of Pseudomonas aeruginosa, MB 5001, was maintained at 4°C on a nutrient agar plate (Difco). A loopful of culture from the solid medium was inoculated to a 25 x 150 mm metal capped tube containing 10 mls of nutrient broth, and incubated at 22.5°C at 300 revolutions/minute. After 24 hours growth, 0.5 mls of the culture was transfered to 100 mls of peptonized milk (100 g/l) in a baffled 1 L flask. Incubation for 40-48 hours at 25°C on a shaker at 250 revolutions/minute was followed by centrifugation of the broth under refrigeration at 10,000 revolutions/minute for 10 minutes. The supernatant was used as the source of enzyme.

Reactions tubes containing 1.8 mls of Pseudomonas aeruginosa supernatant, or Pseudomonas sp. lipase (0.2 mg/ml) or pig liver esterase (2.0 mg/ml) dissolved in 1.8 mls of 45 mM Tris/HCl (pH

7.5) were mixed with 0.2 mls of 40 mM 4-acetyl-simvastatin (Compound (2) above) in methanol. Tubes were incubated in a dry bath at 37°C with no agitation.

Samples (100 µl) taken at intervals beginning at 20 minutes incubation, and ending at 24 hours were diluted in 900 µl of methanol. Analysis of the reaction products was by HPLC using a Perkin-Elmer LC-235 diode array detector with a detection wavelength of 235 nm. A mobile phase mixture of acetonitrile:0.1% phosphoric acid in distilled water (80:20, v/v) was pumped through a Whatman Partisil 5 C8 column (4.6 mm x 25 cm) at 1.5 mls/minute. Standards of simvastatin ammonium salt and simvastatin eluted at 3.2 min. and 4.1 min. respectively. Percent hydrolysis of 4-acetyl simvastatin (R=methyl, R_1 = 2-methyl-2-butyl) to form the open acid form (III) and/or the lactone form (IV) of simvastatin is given in Tables I, II and III.

EXAMPLES 2-4

The following intermediate compounds of formula (II) were prepared following the preparation procedure of Examples 1a to 1C, with the substitution of an equivalent amount of the appropriate acyl halide. The simvastatin hydrolysis products (III) and (IV) were prepared from the intermediate following the procedure of Example 1d. The percent hydrolysis for each intermediate to form the open acid form (III) and/or the lactone form (IV) of simvastatin is found in Table 1.

Example 2 R = n-propyl, R_1 = 2-methyl-2-butyl;
Example 3 R = isopropyl, R_1 = 2-methyl-2-butyl;
Example 4 R = n-pentyl, R_1 = 2-methyl-2-butyl.

TABLE I

Percent formation of hydrolysis products (III + IV) from hydrolysis of simvastatin derivatives (II) by Pseudomonas aeruginosa.

R_1 = 2-methyl-2-butyl

HOUR	R=n-pentyl	R=isopropyl	R=methyl	R=n-propyl
0.0	0.0	0.0	0.0	0.0
1.0	9.0	1.5	2.8	8.3
2.0				13.2
3.0	19.0	3.9	5.8	17.6
4.0				
5.0	28.0	9.1	4.4	25.8
24.0	62.0	22.0	14.9	65.9

TABLE II

Percent formation of hydrolysis products (III + IV) from hydrolysis of simvastatin derivatives (II) by *Pseudomonas* species lipase.
R_1=2-methyl-2-butyl

HOUR	R=n-pentyl	R=isopropyl	R=n-propyl	R=methyl
0.0	0.0	0.0	0.0	0.0
0.3	27.0		53.0	
0.7	51.0		74.0	
1.0	62.0	18.0	75.0	30.6
2.0	78.0	32.0	78.0	49.0
3.0	79.0	44.0	77.0	63.8
4.0		56.0		72.0
5.0	97.0		81.0	

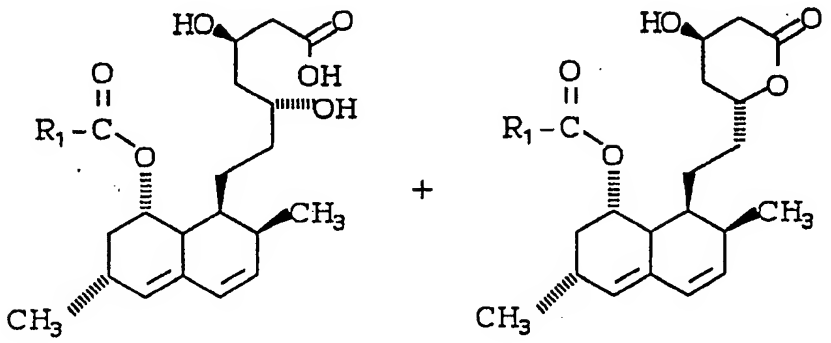
TABLE III

Percent formation of hydrolysis products (III + IV) from hydrolysis of simvastatin derivatives (II) by Pig liver Esterase.
R_1=2-methyl-2-butyl

HOUR	R=n-pentyl	R=isopropyl	R=n-propyl	R=methyl
0.0	0.0	0.0	0.0	0.0
1.0	24.0	42.0	27.0	11.0
2.0		48.0		16.0
3.0	33.0	51.0	41.0	18.0
4.0		59.0		26.0
5.0	34.0		45.0	

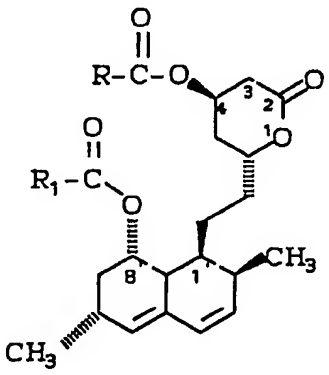
WHAT IS CLAIMED IS:

1. A process for the formation of compounds (III) and (IV):



(III) (IV)

which comprises treating a compound of structural formula (II):



(II)

wherein:

R is C_{1-10}alkyl; and

R_1 is C_{1-10}alkyl;

with an enzyme source selected from the group consisting of

1) Pseudomonas sp. lipase;
2) LPL-80 Lipase;
3) Pig liver esterase;
4) Candida cylindracia Lipase;
5) Supernatant broth of Pseudomonas aeruginosa;

to form compounds (III) and (IV).

2. A process of Claim 1 wherein
R is C_{1-5}alkyl; and
R_1 is C_{1-5}alkyl.

3. A process of Claim 2 wherein R is selected from the group consisting of: methyl, propyl, isopropyl and pentyl; and R_1 is selected from the group consisting of 2-methyl-2-butyl and 2-butyl.

4. A process of Claim 3 wherein R is methyl and R_1 is 2-methyl-2-butyl.

5. A process of Claim 3 wherein R is n-propyl and R_1 is 2-methyl-2-butyl.

6. A process of Claim 3 wherein R is isopropyl and R_1 is 2-methyl-2-butyl.

7. A process of Claim 3 wherein R is n-pentyl and R_1 is 2-methyl-2-butyl.

Patents Act 1977

Examiner's report to the Comptroller under Section 17 (The Search Report)

Application number

9210486.8

Relevant Technical fields

(i) UK Cl (Edition K) C2C (CPN, CBW, CTV)

(ii) Int CL (Edition 5) C07C 37/56

Search Examiner

S I AHMAD

Databases (see over)

(i) UK Patent Office

(ii) ONLINE DATABASE: CAS - ON-LINE

Date of Search

24 JUNE 1992

Documents considered relevant following a search in respect of claims 1 TO 7

Category (see over)	Identity of document and relevant passages	Relevant to claim(s)
	NONE	

Category	Identity of document and relevant passages	Relevant to claim(s)

Categories of documents

X: Document indicating lack of novelty or of inventive step.

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P: Document published on or after the declared priority date but before the filing date of the present application.

E: Patent document published on or after, but with priority date earlier than, the filing date of the present application.

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